HONOURS THESIS
ARIELLA RINK
SUPERVISOR: JOHN HOFFMANN
DEPARTMENT OF BIOLOGICAL SCIENCES:
UNIVERSITY OF CAPE TOWN

TITLE:

Friend or foe? The arrival of *Stator limbatus* (Bruchidae) in South Africa

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ABSTRACT

Stator limbatus is a generalist seed-feeding beetle (Coleoptera: Bruchidae) whose natural range extends from the southern USA to Latin America. Individuals have recently been collected in the Western Cape province of South Africa in seeds of the invasive alien plant species *Acacia mearnsii* and *A. cyclops*. This study conducted a preliminary examination of its oviposition preference, and development on, a number of indigenous and invasive alien *Acacia* species and the invasive alien *Paraserianthes lophantha* (all Fabaceae). Oviposition preference appears to be for larger seeds but *A. ataxacantha* was found to be the only indigenous host suitable for larval development.

INTRODUCTION

Stator limbatus (Horn 1873) is a species of beetle belonging to the family Bruchidae, which together with the families Anthribidae, Cerambycidae, Chrysomelidae, Curculionidae and Scolytidae compose the group Phytophaga, the herbivorous beetles. The family is probably best known for those species which are pests of stored food. In all species the larval cycle is completed within plant seeds, usually of leguminous species. Entire bean crops may be lost to consumption by bruchids if not treated with insecticides or stored properly after harvesting (Morallo-Rejesus 1990 in Kingsolver 2004). In fact only a minority – about thirty species – of the approximately 1200 species of Bruchidae are serious pests, of which nine have attained cosmopolitan distributions due to anthropogenic transport (Kingsolver 2004). *Stator limbatus*’s natural distribution stretches from the southern states of the USA to Mexico and Ecuador. Through anthropogenic transport it has spread to Iran (Boroumand 2010) and the United Arab Emirates (Plant Protection News 2011). It has also been introduced to Hawaii (Kingsolver 2004), where it uses the seeds of the endemic koa *Acacia koa*, destroying up to 90% of the annual seed crop (Swezey 1928 in Stein 1983). Within its native range it is attacked by species of parasitoid wasps (Kingsolver 2004).

Stator limbatus was discovered in South Africa in 2009 when eggs were noted on seeds in samples collected from *Acacia mearnsii* in the Franschoek/Wemmershoek region of the Western Cape (coordinates: 33 51.229   19 02.443) (C.A. Kleinjan, pers. comm.). Upon eclosion, adult beetles were sent by the Plant Protection Research Institute for identification to Dr Delobel, a bruchid systematist based at the Museum National d'Histoire Naturelle in France (Plant Protection News 2011), who confirmed that they were *S. limbatus* and informed the PPRI that the species had also been collected in the United Arab Emirates and Iran (Boroumand 2010) and that there were unconfirmed reports of its presence in Mauritius, Oman, Saudi Arabia and Yemen. Additional eggs were discovered on seeds of *A. cyclops* collected within soil samples taken at Yzerfontein, and live adults were collected on *A. mearnsii* plants in Tokai (J.H. Hoffmann, pers. comm.)

Larval diet

Johnson (1970; in Kingsolver 2004) estimated that 84% of the known host species of Bruchidae are in the family Fabaceae. Many hosts belong to the family Convolvulaceae and, in all, Bruchidae develop in seeds from 32 plant families (Kingsolver 2004). *Stator limbatus* is a generalist, being recorded from seeds of approximately 60 plant species (Kingsolver 2004, Fox et al. 2006). It exhibits a preference for *Acacia* (Fabaceae), of which it is known to use fifteen species (Kingsolver 2004).

Upon hatching, the larva bores through the floor of the egg and thence through the seed coat. For this purpose it is equipped with a toothed dorsal prothoracic plate which provides leverage while exiting the egg and boring through the seed coat (Kingsolver 2004). Once through the seed coat, the larva molts into the second instar and proceeds to feed on the contents of the seed, progressing through a further three instars before entering the pupal stage. In some species, including *S. limbatus*, each individual completes the larval and pupal stages within a single seed, but strategies vary.

Developmental challenges

Bruchid larvae overcome a number of challenges in order to complete their development. They must have the means to successfully enter the seed; they must be able to neutralize, or must be immune to, toxins and other defenses on the seed coat and within the seed; they must be able to develop satisfactorily within one seed; and they must maintain their body moisture content. Seeds usually contain less than 10% water by mass, so larvae depend on water produced from the metabolic breakdown of their food. The metabolic cost of neutralizing toxins and of boring through thick or hard seed coats limits diet breadth; species tend to either be very successful on one heavily-defended host or have less specific counter-defenses (and therefore a lower metabolic cost to development), using a range of hosts but having a higher mortality rate (Johnson and Kistler 1987).

Adult diet

Adults may feed on pollen and nectar when available (Janzen 1980 in Johnson and Kistler 1987), but some species can persist for many generations without their adults feeding at all (Johnson 1981a in Johnson and Kistler 1987). Species which are not obligate feeders as adults still experience benefits from feeding – such as increases in longevity, ovarian and testicular production, oogenesis, oocyte growth, fecundity and frequency of copulation (Pajni and Sood 1975, Leroi 1978, Pesho and Van Houten 1982; all in Johnson and Kistler 1987). Wightman (1978b in Johnson and Kistler 1987) found that unfed *Callosobruchus analis* females lost 10% of their dry body weight during egg production. The longer the period a female *Acanthoscelides obtectus* is prevented from ovipositing, the less fecund she becomes (Bushnell and Boughton 1940, Leroi 1980; both in Johnson and Kistler 1987). It is known that in the species *Zabrotes subfasciatus* and *C. maculatus* – in which the adults are not obligate feeders – newly emerged adults have very high lipid contents (30 – 50%) compared to other insects (less than 10%). This may be specific to the non-feeding species or it may be a buffer against unpredictable pollen and nectar supplies, common to all Bruchidae (Wightman 1978b, Sharma and Sharma 1979b; both in Johnson and Kistler 1987).

Host choice and oviposition guilds

Females preferentially lay eggs on seeds with the fewest eggs, as the probability that a larva develops successfully to adulthood decreases, due to competition for food, with increasing larval density per seed (Horng 1997). Some females are more willing than others to inspect many seeds before coming to a decision. Thus, if a group of females is presented with a very large number of seeds it is likely that a few seeds will remain unused while others will bear large egg loads.

Johnson (1981a in Kingsolver 2004) describes three oviposition guilds. Members of the mature pod guild lay eggs on the exterior of the seed pod and larvae must bore through the pod wall as well as the seed coat to access food. In the mature seed guild, females enter partly dehisced pods or intact pods through holes made by other insects; *S. limbatus* belongs to this guild. Eggs are laid on the exterior of mature seeds so that larvae need only bore through the seed coat. Lastly, species in the scattered seed guild lay eggs on seeds which have fallen from mature, fully dehisced pods.

Invasive alien Acacia species in South Africa

By the advent of the millennium, 548 plant species introduced over the years to South Africa were listed as naturalized or casual (Henderson 2007); by 2010, this had increased to 660 species (Wilson et al. 2013). South Africa's Conservation of Agricultural Resources Act, Act 43 of 1983, recognizes 198 species introduced to the country as declared weeds or invaders (Henderson 2001a, Wilson et al. 2013), of which 36 species belong to the Fabaceae (Henderson 2001a). Approximately 10 million ha of the 122 million ha covered by South Africa is estimated to be colonized by invasive alien species (henceforth referred to as IAPs), consuming an estimated 3.3 billion litres of water per annum (van Wilgen and De Lange 2011).

The most highly invaded biome, in terms of area covered, is the Fynbos (Henderson 2007), and the most widespread IAPs therein are *A. mearnsii, A. saligna, A. cyclops, A. melanoxylon* and *A. longifolia*, as well as *Paraserianthes lophantha*. Species belonging to the Fabaceae are prominent in all biomes in terms of both area covered and species number. Henderson (2007) considers four (*A. mearnsii, A. saligna, A. cyclops and A. dealbata*) of the seventeen species of introduced *Acacia* as amongst the ten most prominent IAPs in the country.

Biological control agents for *Acacia* species and *P. lophantha* have been in use in South Africa since 1982 (Impson et al. 2011). The Australian *Acacias* are particularly prolific seed producers, creating seedbanks many tens of centimetres thick that can persist for years after clearing. Moreover, their seeds are able to germinate after years of dormancy and are stimulated to do so by fire. Thus they are well-suited to the very fires on which many fynbos species also depend for their seeds' germination. Neither mechanical clearing nor fire is adequate in eliminating *Acacias* but seed-feeding insects, imported from Australia may curb their recruitment. Seed-feeding beetles of the genus *Melanterius* have been deployed against *A. longifolia, A. melanoxylon, A. cyclops, A. mearnsii, A. dealbata, A. decurrens, A. saligna, A. pycnantha, A. baileyana, A. podalyriifolia and P. lophantha* (Impson et al. 2011). *Melanterius* beetles are however unreliable in the amount of seed damage they inflict and their impact on the target IAPs has been found to vary from stand to stand and from year to year. The most successful effort has been the introduction of *M. compactus* for the destruction of *A. saligna* seedbanks. This species has been able to destroy more than 90% of the seedbank at most sites (Impson, unpubl. data in Impson et al. 2011), and is being introduced steadily into all areas of *A. saligna* infestation (Impson et al. 2011).

Indigenous Acacias of South Africa

Of the 1200 species of *Acacia* worldwide, approximately fifty are distributed wholly or partly within southern Africa. Unlike the Australian species, African *Acacias* possess thorns and thorn-like structures in defense against large herbivores, and all have bipinnate leaves, while many of the invasive Australian species in the region have simple leaves or phyllodes. The most widespread species in South Africa are: *A. erioloba*, *A. mellifera*, *A. karroo* and *A. natalitia* (Palgrave 2005).

This study

From a conservation point of view, the recent introduction of *S. limbatus* into South Africa is of concern because it is a generalist feeder already known to use a number of *Acacia* species in its natural and extended ranges and it may cause substantial damage on native *Acacias* here. From another perspective, it msay prove a valuable addition to the insect biological control agents that are already deployed against the invasive alien *Acacias* and *P. lophantha*.

This study intends to shed some light on this dilemma by providing an assessment of the relative preferences of *S. limbatus* for, and success on, a representative selection of indigenous and exotic *Acacias*.

METHODS

A description of each of the plant species from which seeds were tested is given in Appendix 1.

Seed and insect material

Seeds of *A. cyclops* bearing *S. limbatus* eggs were collected in soil samples taken at Yzerfontein (J. H. Hoffmann, pers. comm.). These seeds were maintained at room temperature until the adults eclosed from them. These *A. cyclops*-reared beetles were supplied with *A. cyclops* seeds as needed to maintain the population until the commencement of this study (J. H. Hoffmann, pers. comm.). Live adults were collected from *A. mearnsii* in Tokai (J. H. Hoffmann, pers. comm.) and were also maintained at room temperature and supplied with seeds of *A. mearnsii* as needed to maintain the population.

Seeds of *A. mearnsii, A. cyclops* and *P. lophantha* were collected by John Hoffmann, seeds of *A. saligna* were collected from a single tree in Hout Bay, and seeds of *A. ataxacantha, A. caffra, A. nigrescens* and *A. sieberiana var. woodii* were bought from Silverhill Seeds, an indigenous seed company based in Cape Town. All seeds were examined before use and those that were damaged were discarded.

Colony

Beetles reared from *A. cyclops* were transferred to 88-mm diameter petri dishes and provided with a constant supply of seeds to maintain a steady supply of newly-emerged adults throughout the duration of the trials. The rearing dishes were checked regularly to remove spent seeds and dead adults and to provide more seeds when necessary. To obtain adults for choice trials, seeds with eggs were collected from the dishes. Excess eggs were removed from seeds with more than one egg to ensure that there was no crowding and that each larva had a plentiful supply of nutrients. If there was more than one egg that had already hatched then the seed was placed back into the rearing dishes. Seeds from which all excess eggs had been removed were stored in 96- and 24-well microtitre plates, one seed to a well. The lids fit loosely enough that fresh air could enter the wells. While beetles did on occasion escape from their individual wells, the design of the lid is such that they could not escape from the plate and were contained until needed.

The trials

Trials consisted of two-choice tests in which newly-emerged (<24 hours old) females and males from the *A. cyclops* colony were presented with twenty seeds of *A. cyclops* and twenty seeds of a test species. The indigenous test species were *A. ataxacantha, A. caffra, A. nigrescens* and *A. sieberiana var. woodii*. The invasive alien test species were *A. mearnsii, A. saligna* and *P. lophantha*. Trials were at minimum two days long; if by this period no eggs were visible, trials were continued until eggs were noted. A number of male and female beetles were used in every trial in order to maximise the possibility of mating and thence oviposition.

Upon termination of a trial the beetles were removed and kept in a pill bottle with a pierced lid to supply them with fresh air. The seeds were examined for eggs and for each species the egg-load of every seed was noted. Excess eggs were removed so that the successful development of larvae could be attributed only to the species of seed in which they developed and not to inter-larval competition. Each seed was stored in a labelled pill bottle with pierced lid. These were inspected regularly from approximately twenty days after the termination of the trial. The date of eclosion of the beetles that emerged from each seed was noted along with their mass, sex and species of seed in which they developed. Once dead their head capsules were measured along the dorsal axis, from mandible to back edge. The sex and head capsule length of twenty beetles of the *A. mearnsii* colony and twenty beetles from the *A. cyclops* colony were measured to provide an indication of the plasticity of body size depending on host species. All measurements were made at 40x magnification using a Leica dissecting microscope fitted with a graticule.

Seed size

Seed dimensions and mass were measured as indicators of seed size. Variables measured were: mass, width, thickness and length. Measurements were made on ten seeds in a sample for each species. Width, thickness and length were measured using a KRAFFT digital calliper accurate to 0.01mm. Mass was measured on a balance accurate to 0.0001g.

Statistical analysis

Preference for the test species in each trial was calculated as:

$$\text{Preference} = \frac{\text{no. eggs on test sp. seeds in replicate}}{\text{total egg no. in replicate}}$$

Mean preference per test species was plotted in Microsoft Excel 2007. The range of preference for each species was indicated by the highest and lowest preference values obtained. Seed size was analysed with a Principle Components Analysis (PCA) performed in R version 3.0.1 (2013). Data were first standardized, then inputed into a PCA analysis.

The mean preference values were analysed with a three-way Analysis of Variance (ANOVA) test performed in R version 3.0.1 (2013). The ANOVA tested for significant differences between species' mean preference values, considering the number of females in each trial and whether or not food (dissolved yeast) had been supplied in each trial. A pairwise t-test without p-value adjustment was performed to identify the significant differences found.

Body size plasticity was tested using an Analysis of Covariance (ANCOVA) test performed in R version 3.0.1 (2013). This tested whether host species was a significant determiner of body size (for which head capsule length was the proxy) given the sex of the individuals measured. A pairwise t-test without p-value adjustment was performed to identify the significant differences found.

RESULTS

Seed size

Principle Component (PC) 1 accounts for almost two-thirds of the variation in seed dimension and mass, while PC2 accounts for a further 22.5% (Table 1). Axis PC1 (see Table 1) is primarily a measure of mass (0.57) and length (0.55), while PC2 is a measure of thickness (0.89). *Acacia sieberiana var. woodii* seeds vary widely in their PC2 values but have amongst the highest PC1 values, indicating that they are relatively heavy and large in surface area compared to seeds of the other species (Figure 1). *Paraserianthes lophantha* seeds are similar to the former in thickness but are smaller, more circular and lighter. These two species could be considered as the 'thick group'. *Acacia nigrescens, A. ataxacantha* and *A. caffra* score distinctly lower on PC2; these species could be considered the 'thin group'. Lastly, *A. cyclops, A. tortilis, A. mearnsii* and *A. saligna* are intermediate between the former groups in thickness and achieve the lowest PC1 scores. *Acacia nigrescens* seeds have the largest surface area, followed closely by *A. sieberiana var. woodii*; *A. mearnsii* and *A. saligna* seeds have the smallest surface areas. *Acacia ataxacantha* and *A. caffra* score similarly on PC1 but the former is slightly thicker. *Acacia mearnsii* seeds are the most uniform in dimension and mass.

Preference for test species over known host Acacia cyclops

The discrepancy between number of eggs laid on the known host and the number of eggs laid on a test species was highest when the test species was *A. sieberiana var. woodii* (Figure 2;

mean preference score = 0.96); in four of the five replicates of *A. sieberiana var. woodii* eggs were only oviposited on *A. sieberiana var. woodii*. *Paraserianthes lophantha* was preferentially used to almost the same degree (mean preference score = 0.93). All test species, with the exception of *A. mearnsii* and *A. saligna*, were 'preferred' as hosts over *A. cyclops* (Figure 2). The results of the three-factor ANOVA test examining significant differences in mean 'preference' score per species given differing numbers of females per replicate and the fact that beetles in some replicates were provided with food are presented in Table 2. The mean 'preference' score was highly significantly different across species ($F_{6, 5} = 110.911$; $p < 0.001$), while the interaction species:number of females was also significant, albeit less so ($F_{6, 5} = 6.083$; $p < 0.05$). The pairwise comparison displayed in table 3 shows which species were significantly 'preferred'. *Acacia mearnsii* was significantly less 'preferred' over all species except *A. saligna*, which was also significantly less 'preferred' over all species except *A. mearnsii*. *Acacia sieberiana var. woodii* was 'preferred' significantly more than all other species except *A. nigrescens* and *P. lophantha*. Mean 'preference' for *A. caffra* was significantly different from all other species except *A. ataxacantha*.

Plasticity of body size

Nine individuals developed successfully within *A. ataxacantha* on which eggs had been oviposited during the trials. Host species was the only significant determiner ($F_{2, 42} = 36.742$; $p < 0.001$) of beetle size (Table 4). Individuals that had developed within seeds of *A. mearnsii*, *A. cyclops*, and *A. ataxacantha* exhibited significantly different body sizes (Table 5 and see Figure 3). Mean thickness of *A. ataxacantha* and *A. mearnsii* seeds was not significantly different (two-tailed t-test assuming unequal variances; t = 1.47 df=15, p=0.16).

Effect of host species on larval development and lifespan

Total lifespan did not differ greatly in *A. cyclops*-raised and *A. ataxacantha*-raised individuals, although the latter underwent slightly longer periods of larval development and correspondingly shorter adult stages.

Table 1. PCA axis composition. The more variation is accounted for in an axis, the more important are the variables that lie along that axis in distinguishing between species' sizes.

	PC1	PC2	PC3	PC4
Thickness (mm)	0.3113955	0.8869236	-0.007384584	-0.3410937
Width (mm)	0.5295511	-0.37565	-0.589503115	-0.4805713
Length (mm)	0.5477733	-0.2481541	0.782356741	-0.1621168
Mass (g)	0.5679374	0.1033109	-0.200871788	0.7914698
Proportion of Variance	0.7343	0.225	0.02517	0.01554

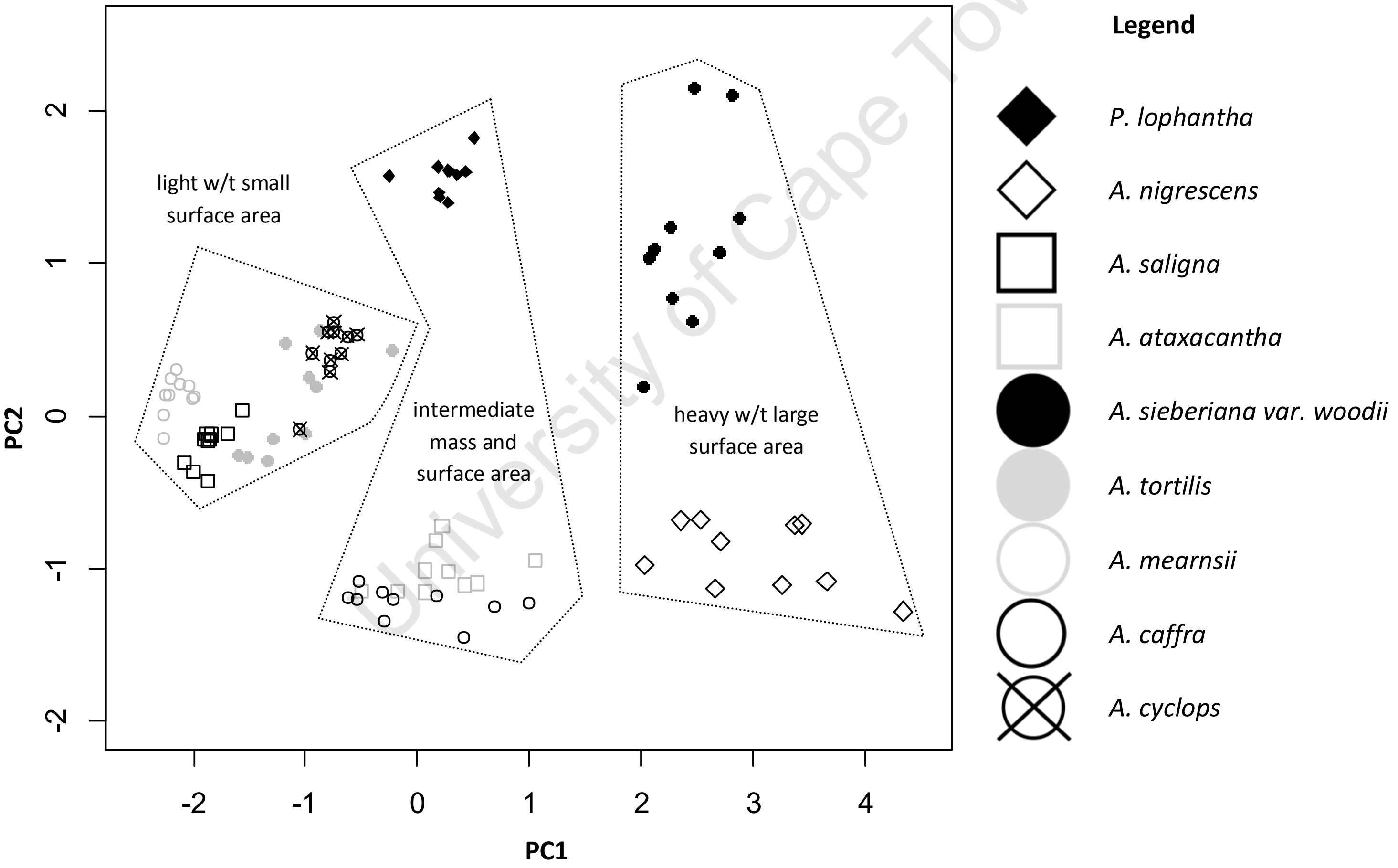

Figure 1. PCA analysis of seed size. Each point represents a seed. PC1 is the most important axis and is primarily a measure of mass (g) and length (mm); PC2 is primarily a measure of thickness (g).

Table 2. ANOVA of mean preference score per species. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘#’ 0.1 ‘~ ’ 1. Only factors coded ‘***’, ‘**’ or ‘*’ significantly determine mean preference scores.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	6	2.8098	0.4683	110.911	<0.001***
Number of females	1	0.0039	0.0039	0.919	0.3818
Fed	1	0.0004	0.0004	0.094	0.7713
Species:number of females	6	0.1541	0.0257	6.083	<0.05*
Species:Fed	2	0.0158	0.0079	1.868	0.2479
Number of females:Fed	1	0.0002	0.0002	0.057	0.8209
Species:Number of females:Fed	1	0.0025	0.0025	0.586	0.4786
Residuals	5	0.0211	0.0042		

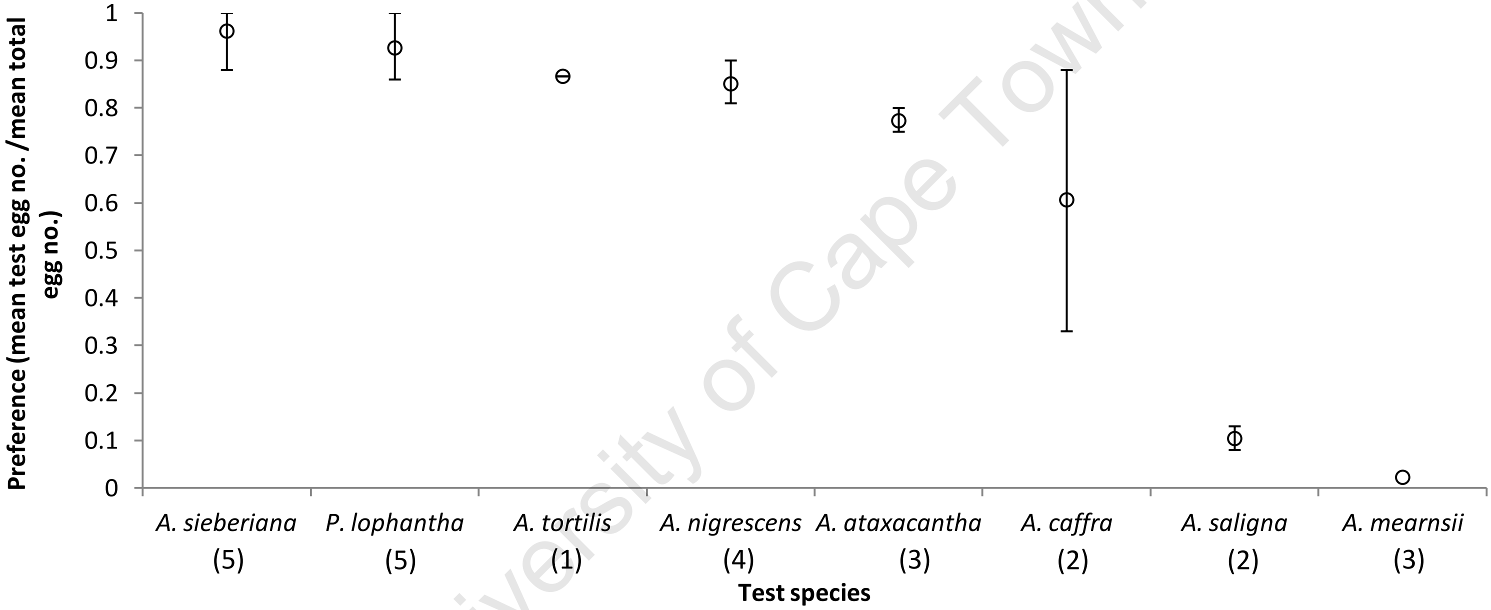

Figure 2. Mean ‘preference’ for each species. o = mean preference score; positive error bar = maximum preference score; negative error bar = minimum preference score. Number below each species name shows number of trial replicates.

Table 3. Pairwise t-test of ANOVA of mean preference score per species. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘#’ 0.1 ‘~ ’ 1. Only species comparisons coded ‘***’, ‘**’ or ‘*’ represent species with significantly different mean preference scores. P-values are unadjusted.

	A. ataxacantha	*A. caffra*	*A. mearnsii*	*A. nigrescens*	*P. lophantha*	*A. saligna*
A. caffra	~					
A. mearnsii	***	***				
A. nigrescens	~	*	***			
P. lophantha	#	**	***	~		
A. saligna	***	***	~	***	***	
A. sieberiana	*	**	***	~	~	***

Table 4. ANCOVA of beetle size plasticity. Host species: *Acacia ataxacantha, Acacia cyclops, Acacia mearnsii*. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘#’ 0.1 ‘~ ’ 1. Only factors coded ‘***’, ‘**’ or ‘*’ are significant determiners of beetle size.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Host	2	2.35E-04	1.17E-04	36.742	<0.001***
Sex	1	1.60E-07	1.60E-07	0.051	~
Host:Sex	2	4.36E-06	2.18E-06	0.683	~
Residuals	42	1.34E-04	3.19E-06		

Table 5. Pairwise t-test of ANCOVA of beetle size plasticity. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘#’ 0.1 ‘~ ’ 1. Only comparisons coded ‘***’, ‘**’ or ‘*’ represent host species supporting significantly different mean beetle sizes. P-values are unadjusted.

	A. cyclops	*A. mearnsii*
A. mearnsii	***	-
A. ataxacantha	***	*

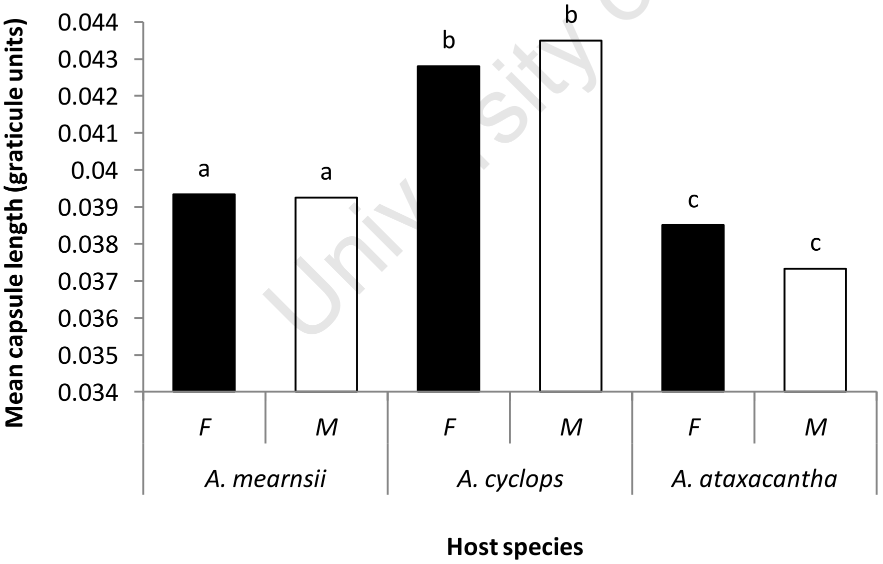

Figure 3. Mean body size of *S. limbatus* raised on different host species. Letters above bars indicate significant differences. F = females; m = males.

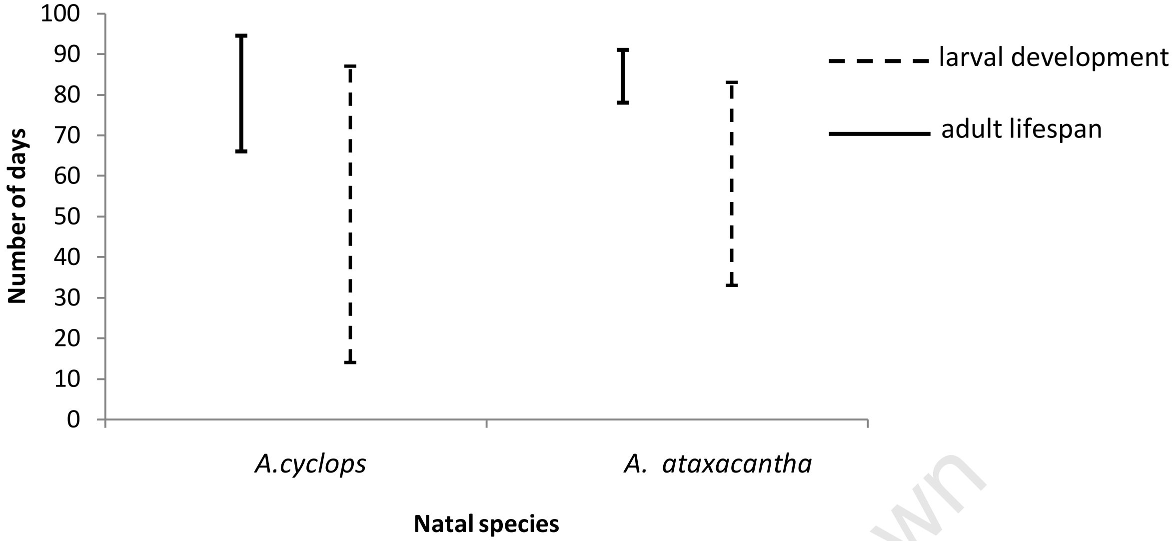

Figure 4. Ranges of duration of larval development and of adult lifespan of *S. limbatus* raised on different host species. Lower and upper line limits correspond to minimum and maximum numbers of days recorded, respectively.

DISCUSSION

Preference

Stator limbatus accepted all the test species for oviposition on at least one occasion but were only able to complete larval development in *A. ataxacantha* seeds. Those species which were most preferred scored highest in the PCA (Figure 1); it appears that oviposition preference is based to some extent on seed size. Greatest preference was given to thick, relatively heavy seeds, and least preference to seeds of *A. saligna* and *A. mearnsii*, which were lighter than those of *A. cyclops*. Females may consider seed size when making oviposition choices because a larger seed would be expected to contain a greater amount of food for larval development. This is an important consideration for *S. limbatus* as its larvae are non-mobile and are thus constrained to develop within the seed on which they were oviposited.

Success

That only *A. ataxacantha* could support larval development suggests that factors other than food availability limit larval development. It was observed that the seeds of *A. nigrescens* and *A. caffra* were coated with fine powder; during a number of trials using these species all beetles died within a matter of hours. When washed and dried to remove this powder, however, they were shown to be highly preferred for oviposition. The ancient Egyptians are known to have protected their grain from insect pests by adding ash powder (Encyclopedia of Insects), which caused desiccation. These seeds, and those of the other unsuitable species, may have also contained substances toxic to *S. limbatus*, or may have been protected with impenetrable seed coats. The relative thinness of *A. ataxacantha* seeds may have limited the allometric growth of larvae, so that although *A. ataxacantha* seeds are larger than *A. cyclops* seeds, larvae feeding therein cannot attain equivalent girth. Thus these beetles begin adult life with lower fat reserves and perhaps, therefore, lower reproductive capacity. Plasticity of body size allows *S. limbatus* to use seeds of a range of sizes; were their body size more constrained they would likely have been less successful at expanding into new territories such as Hawaii and Iran.

Problems

Time-restraints allowed five replicates at most per test species; it would have been preferable to have performed at least this number of replications. A greater number of replications would have been analyzed had many trials not ended without oviposition, despite each being performed with at least one female and one male beetle. A more representative sample of indigenous and invasive alien species would have been more informative of the potential breadth of *S. limbatus*' host use in South Africa. Daily monitoring of eclosion was not always possible. Thus the beetles used in the trials may not have been consistent in age. Irregular monitoring also impeded the accurate measurement of larval development time and adult lifespan across host species. The number of females per trial was not fixed due to difficulty in identifying beetle sex; this was found to have a significant effect on oviposition preference. Of concern too is the decision to test preference in two-choice trials. In reality, a female may only be presented with one *Acacia* species on which to oviposit, and thus may more readily oviposit on less preferred species in no-choice situations. It is apparent that populations of *S. limbatus* are supported on *A. mearnsii* in the Franschoek/Wemmershoek region and in Tokai, but one might surmise that this species is unsuitable for larval development given that females in the two-choice trials barely oviposited on its seeds. Were *S. limbatus* being tested as a potential biological control agent to introduce to the country, no-choice testing of all potential indigenous host species would be imperative.

The long-term impacts of *S. limbatus* in the country are at present uncertain. It may remain distributed only within the dense stands of *A. cyclops* and *A. mearnsii* in the Western Cape where it could perform a complementary role to that of the biological control agents intentionally introduced to manage these invasive alien species. The country's indigenous *Acacia* species are not widespread within the Western Cape. *Acacia ataxacantha* does not occur in the Western Cape at all (see Appendix I) but given the ease with which *S. limbatus* has spread globally it is likely that individuals will reach populations of this and other indigenous *Acacias*. Future studies on the host range of *S. limbatus* in South Africa could consider a more comprehensive selection of indigenous and invasive *Acacia* species, analyzing seeds for toxins that would prevent larval development. Research could identify those aspects of *A. ataxacantha* seeds that are amenable to larval development and search for other species whose seeds possess these 'hospitable qualities.' Field studies should be conducted to assess the current dynamics of the South African *S. limbatus* population. Factors such as the extent of damage to *A. saligna* and *A. mearnsii* seedbanks, rate of population spread and use of novel hosts – indigenous and invasive – should be ascertained.

Thus a more comprehensive study, including a wider range of indigenous and invasive exotic *Acacia* species tested in no-choice as well as two-choice trials with greater replication is essential to a more accurate understanding of *S. limbatus*' impacts in the country.

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APPENDIX I

Invasive alien species

Acacia cyclops is an Australian wattle introduced to the country during the 19th century for its use as a dune stabilizer. It has become widespread throughout the Western Cape and its distribution extends along the West and South coasts (Henderson 2001b). Its seeds are stimulated to germinate by fire and so this species has become a major concern in the fire-regulated fynbos biome. Its impediment to the natural movement of coastal sands is also of concern. Seed production was estimated by Milton and Hall (1981; in Holmes et al. 1987) to amount to approximately 3 000 seeds per m^2 of canopy per year. The waterproof seed testa ensures that many seeds remain dormant, allowing large seedbanks to accumulate. Seeds may remain viable for up to 50 years, and those deepest underground (usually oldest) tend to germinate most readily in response to treatment (Milton and Hall 1981 in Richardson and Kluge 2008).

Acacia mearnsii is also an Australian wattle, introduced for the harvesting of its bark, which is rich in tannins (Stirton 1978). It thus cannot be managed by any means that reduce the economic value of those individuals in plantations; seed-feeding *Melanterius* beetles have been introduced to stop its spread outside of these managed plantations without affecting growth or quality within the plantations (Impson et al. 2011). It is the most extensive invasive plant species along South Africa's rivers (Henderson 2001a, Holmes et al. 2005 in Richardson and Kluge 2008). It is able to resprout after fires or mechanical clearing (Richardson and Kluge 2008), and old seeds sprout as readily as young ones (Pieterse and Boucher 1997 in Richardson and Kluge 2008). *Acacia mearnsii* is established in the Western Cape and along the East Coast, through the Eastern Cape, Kwa-Zulu Natal, parts of the Free State, Mpumalanga and the Northern Province.

Acacia saligna originates from south-west Australia, and, like *A. cyclops*, it was introduced during the early 19th century to bind sand dunes. An early project was to stop the movement of sand across the Cape Flats. A mixture of the city's refuse and seeds of exotic species, including *A. saligna* was spread over the area (Stirton 1978). It is distributed throughout the Western Cape and is able to produce 10 000 seeds per m^2 of canopy per year (Milton and Hall 1981 in Holmes et al. 1987). Seed inviability is extremely low - only 1% (Milton and Hall 1981 in Richardson and Kluge 2008).

Paraserianthes lophantha was introduced to South Africa in 1833 from western and south-western Australia (Stirton 1978) as an ornamental plant and source of honey (Henderson 2001b). It has since spread along the South coast through the Western Cape and Eastern Cape.

Indigenous species

Acacia sieberiana var. woodii is distributed within Kwa-Zulu Natal, Mpumalanga, Gauteng and the Northern Province on well-drained soils in a variety of habitats. Its seed pods remain indehiscent until some time after falling from the tree (Smit 1999).

Acacia nigrescens is distributed within Kwa-Zulu Natal, Mpumalanga, the Northern Province and the North West Province on non-sandy soils and is fire- and drought-resistant but sensitive to cold. This species is known to occur on occasion in monospecific groups. Its seed pods are borne in bunches and dehisce while still on the tree (Smit 1999).

Acacia caffra is widespread throughout South Africa and other regions of Southern Africa. Populations exist in small regions of the north-western Western Cape and southern Western Cape; mostly, however, this species is distributed in a thick band running along the country's East Coast, through the Eastern Cape and Kwa-Zulu Natal. Its distribution continues through Mpumalanga and most of the Northern Province, extending into the North West Province. It is tolerant of a range of environmental conditions, and is fire-tolerant. Its seed pods are borne in bunches and dehisce while on the tree (Smit 1999).

Acacia ataxacantha's distribution extends from the northern Eastern Cape into much of Kwa-Zulu Natal, through Mpumalanga and into Gauteng and the Northern Province. Unlike *A. caffra, A. nigrescens and A. sieberiana var. woodii*, this species tends to grow as a shrub or small tree, forming thicket. The seed pods are borne in bunches and dehisce while on the tree (Smit 1999). It is the only known indigenous *Acacia* species to be invasive within South Africa (Theron 1978 in Nyoka 2003).

Acacia tortilis is found in Kwa-Zulu Natal, northern Mpumalanga, all of the Northern Province and most of Gauteng, the eastern North West Province and its range extends into the eastern Northern Cape and western Free State. This species favours dry conditions, is a pioneer species in disturbed areas, and is cold-tolerant (Smit 1999). Its high-protein pods are used as livestock fodder (Hines and Eckman 1993). The seed pods are indehiscent, bursting open when sufficiently dry and thus dispersing seeds away from the mother plant (Orwa et al. 2009).